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(54) **Pharmaceutical compositions comprising mannose binding lectin**

(57) The present invention relates to pharmaceutical compositions comprising MBL and/or MBL variants. In particular the invention relates to pharmaceutical compositions comprising at least 200 µg/ml protein containing material, wherein mannan binding lectin (MBL) and/or MBL variants constitutes at least 35% (w/w) of the total protein; or to compositions comprising at least 400 µg/ml mannan binding lectin (MBL) and/or MBL variants. In addition the invention relates to pharmaceutical compositions comprising MBL and/or MBL variants and

divalent cations. The invention also describes methods of preparing said compositions.

The pharmaceutical compositions according to the invention may for example be used in methods of treatment of a number of different clinical conditions including infections. Uses of the compositions for preparation of medicaments for treatment of a clinical condition are also described.

**Description****Field of invention**

5 [0001] The present invention relates to pharmaceutical compositions comprising mannose binding lectin. Furthermore, the invention relates to methods of treatment of a clinical condition using said pharmaceutical compositions and to use of the pharmaceutical compositions for the preparation of a medicament.

**Background of invention**

10 [0002] MBL is a protein of the collectin family and characterised by an oligomeric structure of subunits. Each subunit consists of 3 identical polypeptides each consisting of a calcium-dependent, C-type carbohydrate-recognition domain (CRD), attached to a collagenous rod.

15 [0003] The number of subunits in an MBL molecule is varying (Lipscombe RJ, et al, 1995), but it has been suggested that the biologically active polypeptide is an oligomer consisting of more than three subunits. Naturally occurring MBL in plasma comprises mainly oligomers of more than three subunits, and in addition denatured and structurally impaired protein forms leading to bands on for example SDS gels between the dominating MBL bands corresponding to the higher oligomers.

20 [0004] Recombinantly produced MBL reveals an oligomer variation similar to plasma-derived MBL (Vorup-Jensen T et al., 2001). However, usually recombinantly produced MBL has a higher content of low-mass forms than do plasma derived MBL. Low-mass forms of MBL include for example single polypeptide chains, single subunits, and dimeric subunits.

25 [0005] MBL is structurally related to the C1q subcomponent of component C1 of complement, and it appears that MBL activates the complement system via associated serine protease termed MASPs (Matsushita, M., 1992) or p100 (Ji, Y-H. et al., 1993), which are similar to the C1r and C1s components of the classical pathway. The MBL complement activation pathway is called the MBLection pathway. According to the mechanism postulated for this pathway, MBL binds to specific carbohydrate structures found on the surface of a range of microorganisms including bacteria, yeast, parasitic protozoa and viruses (Turner, M.W, 1996), and its antimicrobial activity results from activation of the terminal, lytic complement pathway components (Kawasaki, N et al., 1989) or promoting phagocytosis (Kuhlman, M et al., 1989).

30 [0006] Reportedly, the level of MBL in plasma may be genetically determined (Sumiya, M et al., 1991, Lipscombe, R.J. et al., 1992, Madsen H.O. et al. 1994). MBL deficiency is associated with susceptibility to frequent infections with a variety of microorganisms in childhood (Super, M et al., 1989, Garred, P. et al., 1995), and possibly, in adults (Garred, P. et al., 1995, Summerfield, J.A. et al. 1995). Recent information associates MBL deficiency with HIV infection and with more rapid death following development of AIDS (Nielsen, S.L et al., 1995, Garred, Petal., 1997). MBL binds to the a galactosyl form of IgG (GO), which is found at elevated concentrations in rheumatoid arthritis patients, and then activates complement (Malhotra, R et al., 1995). MBL deficiency is also associated with a predisposition to recurrent spontaneous abortions (Kilpatrick, D.C et al., 1995), and also to development of systemic lupus erythematosus (Davies, E.J et al., 1995).

40 [0007] MASP-1 (MBL-associated serine protease 1) is a serine protease similar in structure to C1r and C1s of the complement pathway although it has a histidine loop structure of the type found in trypsin and trypsin-like serine proteases. MASP-1 has been found to be involved in complement activation by MBL. A cDNA clone encoding MASP-1 has been reported that encodes a putative leader peptide of 19 amino acids followed by 680 amino acid residues predicted to form the mature peptide.

45 [0008] MASP-2 (MBL-associated serine protease 2) (Thiel, S et al., 1997) is a serine protease similar in structure to C1r and C1s of the complement pathway. Like these, and contrary to MASP-1, it has no histidine loop structure of the type found in trypsin and trypsin-like serine proteases. MASP-2 has been found to be involved in complement activation by MBL. MASP-3 shows some homology with MASP-1 and MASP-2 and the two C1q-associated serine proteases, C1r and C1s.

50 [0009] MBL purified from serum has previously been used in a pharmaceutical formulation furthermore containing human serum albumin to treat two MBL deficient individuals and said MBL treatment resulted in significant reduction of infections (Valdimarsson et al., 1998). In addition, various other pharmaceutical MBL formulations have been described in the prior art:

[0010] Valdimarsson et al. describes infusion of a solution containing 200 µg/ml MBL in 0.15 M NaCl and 1% w/v (= 10 mg/ml) human serum albumin.

55 [0011] US 5,270,199 describes an MBL formulation comprising 5 to 100 µg/ml MBL and/or MBL fragment.

[0012] WO 99/64453 describes various MBL formulations comprising MBL and a stabilising agent. As preferred example is mentioned a formulation comprising 300 to 400 µg/ml MBL in PBS with 0.5% w/v albumin (= 5 mg/ml).

[0013] However, these MBL formulations comprise either low amounts of MBL and/or high concentration of other

proteins.

# Summary of invention

**[0014]** However, there is a need for MBL formulations, comprising stable MBL, in particular such formulations comprising reduced amounts of protein containing stabilisers, such as for example reduced amounts of human serum albumin.

**[0015]** Almost all pharmaceutical formulations comprising proteins or polypeptides as the active ingredient do also comprise one or more stabilisers of said protein or polypeptide. In particular, said stabilisers may be detergents or protein containing stabilisers. For many purposes however, it is desirable to omit or reduce the amount of different additives, such as stabilisers for use in pharmaceutical compositions, in particular the amount of protein containing stabilisers, however frequently reduction of protein containing stabilisers results in reduced stability of the active protein component.

**[0016]** The inventors have developed methods for producing highly concentrated MBL and surprisingly, the inventors have shown that MBL formulations comprising high MBL concentrations may be sufficiently stable to be used in pharmaceutical formulations even if no or only a reduced amount of protein containing stabiliser is added.

**[0017]** Accordingly, it is a first objective of the present invention to provide pharmaceutical compositions comprising pharmaceutically acceptable additives and

a) at least 200 µg/ml protein containing material, wherein mannan binding lectin (MBL) and/or MBL variants constitutes at least 35% (w/w) of the total protein; or

b) at least 400 µg/ml mannan binding lectin (MBL) and/or MBL variants

**[0018]** It is a second objective of the present invention to provide pharmaceutical compositions comprising pharmaceutically acceptable additives; and mannan binding lectin (MBL) and/or MBL variants; and at least one divalent cation.

**[0019]** It is a third objective of the present invention to provide a process for preparation of said pharmaceutical compositions, comprising the steps of

a) Providing mannan binding lectin (MBL) and/or MBL variants

b) Mixing said MBL and/or MBL variants with pharmaceutical acceptable additives and optionally with divalent cations

c) And thereby obtaining said pharmaceutical compositions

**[0020]** It is a further objective of the present invention to provide methods of treatment of a clinical condition in an individual in need thereof, comprising administration of said pharmaceutical compositions. The treatment may be selected from the group consisting of curative, ameliorating, relieving and preventive treatment and supplement treatment.

**[0021]** It is a still further objective of the present invention to provide use of the above mentioned composition, for the preparation of a medicament for the treatment of a clinical condition in an individual in need thereof.

**[0022]** It is a furthermore an objective of the present invention to provide a medicament for the treatment of a clinical condition in an individual in need thereof comprising a composition as described above.

**[0023]** It is furthermore an objective of the present invention to provide methods of diagnosis involving administration of the pharmaceutical compositions mentioned herein above or to provide uses of said pharmaceutical compositions for diagnostic purposes.

## Short description of figures

**[0024]** Figure 1 illustrates the C4 activation data for 1 mg/ml MBL formulation samples stored 12 months at +4 and -20 compared to the reference MBL.

**[0025]** Figure 2 illustrates the SEC chromatograms of the reference standard in top followed by 1 mg/ml stability sample kept at +4 degree and at -20 degree. A small front peak is detected in the sample kept chilled corresponding to 1% of the total MBL content. Figure 3 illustrates mass spectrometry data. Similar peaks for the reference and the 12 months MBL samples indicate no changes in MBL polypeptide chain during storage.

## Short description of sequence listing

SEQ ID NO:1: Human MBL protein sequence

SEQ ID NO:2: Human MASP-1 protein sequence

SEQ ID NO:3: Human MASP-2 protein sequence

SEQ ID NO:4: Human MASP-3 protein sequence

## Detailed description of the invention

MBL and MBL variants

[0026] The present invention i.a. provides pharmaceutical compositions comprising MBL and/or MBL variants. The pharmaceutical compositions should in general comprise a high MBL concentration, for example the composition may comprises at least 200 µg/ml, such as at least 250 µg/ml, for example at least 300 µg/ml, such as at least 500 µg/ml, for example at least 750 µg/ml, such as at least 1000 µg/ml, for example at least 1300 µg/ml MBL and/or MBL variants.

[0027] Accordingly, the composition may comprise in the range from 200 to 10,000 µg/ml, such as from 200 to 5000 µg/ml, for example from 200 to 3000 µg/ml, such as from 200 to 2000 µg/ml, for example from 200 to 1500 µg/ml MBL and/or MBL variants.

[0028] In embodiments of the present invention wherein the pharmaceutical compositions comprises a divalent cation it is preferred that said compositions comprises at least 10 µg/ml, such as at least 25 µg/ml, for example at least 50 µg/ml, such as at least 100 µg/ml, for example at least 150 µg/ml, such as at least 200 µg/ml, such as at least 250 µg/ml, for example at least 300 µg/ml, such as at least 500 µg/ml, for example at least 750 µg/ml, such as at least 1000 µg/ml, such as at least 1500 µg/ml, for example at least 2000 µg/ml, such as at least 2500 µg/ml, for example at least 3000 µg/ml, such as at least 3500 µg/ml, for example at least 4000 µg/ml, such as at least 5000 µg/ml, for example at least 6000 µg/ml, such as at least 7000 µg/ml, for example at least 8000 µg/ml, such as at least 9000 µg/ml, for example at least 10000 µg/ml MBL and/or MBL variants.

[0029] The concentration of MBL should in general be determined by determining and/or calculating the weight of the amino acids constituting MBL. In general, the weight is calculated to correspond to the non-glycosylated form of MBL. Hence in general the weight of MBL does not include any glycosylation, and it does not include water content. In one embodiment the concentration of MBL may be determined using an amino acid analysis as described herein below.

[0030] Estimation of MBL Concentration by Amino Acid Analysis:

[0031] Not all amino acids might be estimated in an amino acid analysis - however, usually the mass of the non-detectable amino acids might easily be estimated from the composition of the detected amino acids when the concentration of a pure or rather pure MBL solution is determined.

[0032] The estimation of MBL concentration may be performed according to a number of different methods. Preferably, one of the two methods described herein below may be employed, more preferably method 2 described herein below may be employed.

[0033] The amount of carbohydrate is usually not estimated, i.e. the stated protein concentration will usually only represent the peptide content alone. However, it is possible to determine the total mass of peptide+carbohydrate from the amount of amino acids.

[0034] Non-detectable amino acids:

When estimating MBL, non-detectable amino acids are

- \* Tryptophane (Trp is hydrolyzed in acid); 3 Trp are present in the polypeptide chain
- \* Histidine (His is co-eluting with Tris); only one His is present in the polypeptide chain
- \* Hydroxylysine (Hyl is not applied during calibration); max 4 Hyl might be present in the polypeptide chain
- \* Hydroxyproline (Hyp); max 8, typically 4, Hyp are present in the polypeptide chain

Detectable - but uncertainly estimated - amino acids are

- \* Lysine (Lys are partly hydroxylized); 15-19 Lys are present in the polypeptide chain; max 4 Lys might be hydroxylated
- \* Proline (Pro are partly hydroxylized); 10-18 Pro are present in the polypeptide chain; max 8 Pro might be hydroxylated.

Method 1:

[0035] Moles of detectable amino acids and moles of non-detectable amino acids in the analyzed sample:

[0036] Non-detectable amino acids = Trp, His, Hyp and Hyl: It is assumed that 4 Hyp, 4 Hyl, 14 Pro, and 15 Lys are present in average.

$$\text{NonDetectedMoles} = [\text{Trp}] + [\text{His}] + [\text{Hyp}] + [\text{Hyl}] = 3 + 1 + 4 + 4 = 12$$

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CorrectionFactor for NonDetectedMoles = AllMoles/DetectedMoles = All-

$$\text{-Moles}/(\text{AllMoles}-\text{NonDetectedMoles}) = 228/(228-12) = 1.0556$$

Non-detectable amino acids = Trp, His, Hyp and Hyl:

$$\text{NonDetectedMass} = [\text{MTrp}] + [\text{MHis}] + [\text{MHyp}] + [\text{MHyl}] =$$

$$3 \cdot 186.21 + 1 \cdot 137.14 + 4 \cdot 113.11 + 4 \cdot 144.17 = 1725.89$$

DetectedMass =

$$[\text{MAssx}] + [\text{MThr}] + [\text{MSer}] + [\text{MGlx}] + [\text{MPro}] + [\text{MGly}] + [\text{MAla}] + [\text{MCys}] + [\text{MVal}] + [\text{MMet}] + [\text{Mlle}] + [\text{MLeu}] + [\text{MTyr}] + [\text{MPhe}] + [\text{MLys}] + [\text{MArg}] =$$

$$25 \cdot 115.09 + 15 \cdot 101.11 + 13 \cdot 87.08 + 27 \cdot 129.12 + 14 \cdot 97.12 + 31 \cdot 57.05 + 16 \cdot 71.08 + 7 \cdot 103.1$$

$$5 + 10 \cdot 99.13 + 2 \cdot 131.20 + 8 \cdot 113.16 + 17 \cdot 113.16 + 1 \cdot 163.18 + 9 \cdot 147.18 + 15 \cdot 128.17 + 6 \cdot 156$$

$$19 = 22429.93$$

CorrectionFactor for NonDetectedMass = AllMass/DetectedMass = (Detected-

$$\text{-Mass} + \text{NonDetectedMass}) / \text{DetectedMass} = 1.0769$$

Method 2:

**[0037]** Non-detectable amino acids = Trp, His, Pro/Hyp and Lys/Hyl: It is assumed that 4 Hyp, 4 Hyl, 14 Pro, and 15 Lys are present in average.

$$\text{NonDetectedMoles} = [\text{Trp}] + [\text{His}] + [\text{Pro/Hyp}] + [\text{Lys/Hyl}] = 3 + 1 + 18 + 19 = 41$$

CorrectionFactor for NonDetectedMoles = AllMoles/DetectedMoles = All-

$$\text{-Moles}/(\text{AllMoles}-\text{NonDetectedMoles}) = 228/(228-41) = 1.2193$$

**[0038]** Mass of detectable amino acids and mass of non-detectable amino acids in the analyzed sample:

**[0039]** For each amino acid, mass is estimated as moles times molar mass (corrected for the missing peptid bond water).

$$\text{For example: } [\text{MThr}] = [\text{Thr}] \cdot (119.12 - 18.01) = [\text{Thr}] \cdot 101.11$$

Non-detectable amino acids = Trp, His, Pro/Hyp and Lys/Hyl:

NonDetectedMass =

$$[\text{MTrp}] + [\text{MHis}] + [\text{MPro}] + [\text{MHyp}] + [\text{MLys}] + [\text{MHyl}] =$$

$$3 \cdot 186.21 + 1 \cdot 137.18 + 97.12 + 19 \cdot 128.17 = 4879.16$$

$$\text{DetectedMass} =$$

$$[\text{MAx}] + [\text{MThr}] + [\text{MSer}] + [\text{MGlx}] + [\text{MGly}] + [\text{MAla}] + [\text{MCys}] + [\text{MVal}] + [\text{MMet}] + [\text{Mlle}] + [\text{MLE}$$

$$\text{u}] + [\text{MTyr}] + [\text{MPhe}] + [\text{MArg}] =$$

$$25 \cdot 115.09 + 15 \cdot 101.11 + 13 \cdot 87.08 + 27 \cdot 129.12 + 31 \cdot 57.05 + 16 \cdot 71.08 + 7 \cdot 103.15 + 10 \cdot 99.1$$

$$3 + 2 \cdot 131.20 + 8 \cdot 113.16 + 17 \cdot 113.16 + 1 \cdot 163.18 + 9 \cdot 147.18 + 6 \cdot 156.19 = 19147.70$$

$$\text{CorrectionFactor for NonDetectedMass} = \text{AllMass} / \text{DetectedMass} = (\text{Detected}$$

$$-\text{Mass} + \text{NonDetectedMass}) / \text{DetectedMass} = 1.2548$$

Comparison of evaluation method 1 and method 2:

**[0040]** Method 1 is based on estimates of appr. 95% of the amino acids, while Method 2 is based on estimates of only appr. 75% of the amino acids.

**[0041]** In general, all performed amino acid content estimations are calculated by both methods, and no significant difference in results are usually observed. Method 2 makes no assumptions on content of Hyl and Hyp, and is therefore preferred.

**[0042]** For certain purposes the concentration of MBL is given in molar rather than as weight/volume. Because MBL may be present as oligomer comprising different amounts of subunits it is difficult to define 1 mole of MBL. Within the scope of the present invention it is preferred that 1 mole MBL refers to 1 mole of individual MBL polypeptide chains. Said individual MBL polypeptide chains may be non-glycosylated, partly glycosylated or fully glycosylated. It is preferred that molarity of MBL is calculated anticipating that MBL is not glycosylated.

**[0043]** The molecular weight of non-glycosylated individual MBL polypeptide chains is 24800 Da. The molecular weight of fully-glycosylated individual MBL polypeptide chains is 25900 Da. The molecular weight of the most commonly occurring form of glycosylated individual MBL polypeptide chains is 25500 Da.

**[0044]** Hence, dependent on the glycosylation status of MBL 1 mg/ml MBL corresponds to in the range of 38 to 41  $\mu\text{M}$ , preferably in the range of 38.6 to 40.3  $\mu\text{M}$ . For most practical applications 1 mg/mL MBL corresponds to around 39.2  $\mu\text{M}$ . However, 1 mg/ml unglycosylated MBL corresponds to around 40.3  $\mu\text{M}$ , whereas 1 mg/mL fully glycosylated MBL corresponds to around 38.6  $\mu\text{M}$ .

**[0045]** The above calculations refer to MBL of SEQ ID No. 1.

**[0046]** However, it is apparent that the methods may be adapted to estimate the concentration of MBL fragments and/or variants.

**[0047]** For many purposes it is desirable that the pharmaceutical composition comprises a reduced amount of proteins other than MBL (see herein below).

**[0048]** Accordingly, pharmaceutical composition are preferred, wherein MBL or MBL variants constitutes at least 35% (w/w), such as at least 40% (w/w), for example at least 50% (w/w), such as at least 60% (w/w), for example at least 70% (w/w), such as at least 80% (w/w), for example at least 90% (w/w), such as at least 95% (w/w), for example at least 98% (w/w), such as at least 99% (w/w), for example at least 99.5% (w/w), such as at least 99.8% (w/w) of the total protein.

**[0049]** In one embodiment of the invention MBL constitutes essentially all of the total protein. The term "essentially all" is meant to encompass "all detectable", preferably all protein detectable by coomassie brilliant blue staining.

**[0050]** In another embodiment the pharmaceutical composition may comprise commonly used protein additives, for example proteins acting as stabilisers or protein containing stabilisers. Proteins useful as stabilisers includes for example human serum albumin or other kinds of serum albumin. Furthermore, protein containing stabilisers may be provided in crude human serum or fractions thereof.

**[0051]** It is in general important for the stability of protein containing pharmaceutical formulations, that they comprise

a suitable concentration of proteins. In general, if the concentration of the protein of interest is low, there is a significant risk of unspecific adhesion of the protein of interest to surfaces of a storage container or the like. Furthermore, protein-protein interactions stabilises the 3 dimensional structure of proteins and higher protein concentration may therefore reduce denaturation. On the other hand, protein solutions comprising a high concentration of the protein of interest are in general not stable, because aggregation of said protein may occur at a *faster* rate (Frokjaer and Hovgaard, p. 102, Wang, 1999). To overcome the above mentioned problems it is therefore common to add stabilisers, for example protein containing stabilisers, such as albumin (Akers et al., 2000, pp. 166-167) or for example detergents to pharmaceutical formulations comprising proteins.

[0052] It is however not always desirable to add stabilisers to a pharmaceutical composition. Commonly used protein containing stabilisers such as albumin are usually purified from serum, which is undesirable because of the risk of transferring pathogens from the serum donor. In addition, it is frequently desirable to reduce the number of additives added to a pharmaceutical composition in order to keep the production as simple as possible. Fewer production steps are easier to handle, reduces costs and also reduces the risk of protein loss and degradation. Hence, it is frequently preferred that no protein stabilisers are added, for example that no protein containing stabilisers and no detergents are added.

[0053] Surprisingly, in one embodiment the present invention discloses a pharmaceutical formulations comprising MBL, wherein said pharmaceutical composition does not comprise any protein stabilisers, i.e. the formulation does not comprise other detectable protein than MBL and the formulation does not comprise any detergent.

[0054] Hence, in one preferred embodiment of the invention however, MBL essentially constitutes all of the total protein of the pharmaceutical composition. In particular, it may be preferred that the composition does not comprise proteins purified from serum or from other human or animal body samples. Because of the risk of transferring viruses or other pathogenic agents from the donor, it is frequently desirable to administer pharmaceutical compositions, which do not comprise compounds purified from serum or from other human or animal body samples.

[0055] Accordingly, in one preferred embodiment of the present invention the pharmaceutical composition does not comprise proteins or protein containing material purified from serum or from other human or animal body samples. In that embodiment it is preferred that all or essentially all protein containing material is prepared using recombinant methods.

[0056] In one embodiment of the present invention the MBL or MBL variants may be modified in order to further enhance stability. Accordingly, it is contained within the present invention that MBL or MBL variants may be PEGylated, acylated or modified in other ways to increase the half-life of MBL or MBL variants in vivo.

[0057] The term MBL and MBL variants is meant to include any form of MBL or any functional homologue thereof. In particular, MBL and MBL variants may be proteins comprising at least one polypeptide comprising the amino acid sequence identified as SEQ ID 1 or a functional homologue thereof.

[0058] MBL according to the present invention may comprise one or more subunits. Each MBL subunit normally consists of 3 individual polypeptides, preferably each individual polypeptide comprises an amino acid sequence as identified by SEQ ID 1 or a fragment thereof of a functional homologue thereof. For example MBL may be monomers, dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers, decamers, 11-mers, 12-mers of subunits or MBL may comprise even more than 12 subunits. Preferably, MBL comprises more than two subunits.

[0059] MBL to be used with the present invention may be derived from any suitable source, for example MBL may be naturally occurring MBL or MBL may be recombinantly produced MBL. When recombinantly produced MBL is used with the present invention it is preferred that said recombinantly produced MBL has a size distribution profile that is similar to naturally occurring MBL. Accordingly, it is preferred that at least 50%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 99% MBL has a apparent molecular weight >200 kDa when determined by non-reducing SDS-PAGE.

[0060] According to the invention, MBL may be human MBL or MBL of other animal species, in which the immune system in this respect is acting like the human immune system, for example, but not limited to MBL from chimpanzees and rhesus monkeys.

[0061] In one embodiment of the present invention MBL is purified naturally occurring human serum MBL. In another embodiment of the invention MBL has been recombinantly produced. It is preferred that the MBL has been recombinantly produced.

[0062] Examples of preferred methods of producing recombinant MBL are described in PCT application WO00/70043, which is hereby incorporated herein by reference. In particular, recombinant MBL to be used in this invention may be prepared as is described in example 1 of PCT application WO00/70043. In the example the recombinant MBL is prepared by the use of an expression vector comprising sequences from the human MBL gene. MBL may also be produced as disclosed in example 1 herein below.

[0063] In order to obtain pharmaceutical compositions comprising very concentrated MBL and/or MBL variant, it may be required to concentrate an concentrated MBL and/or MBL variant composition. Concentration may be done by any conventional method, for example by using a filtration device and exposing it to a centrifugal force. However, for con-

centration of large protein quantities other methods may be preferred.

[0064] The invention also concerns the pharmaceutical compositions comprising MBL variants and methods using same. MBL variants may for example be functional homologues of MBL (see for example herein below). The isolated MBL peptide including any functional homologues thereof, may in one embodiment comprise at least 80 amino acid residues, such as at least 100 amino acid residues, such as at least 150 amino acid residues, such as at least 200 amino acid residues, for example at least 220 amino acid residues, such as at least 250 amino acid residues.

#### Other protein

[0065] The pharmaceutical composition according to the invention may comprise other proteins than MBL.

[0066] For example the composition may comprises at least 300 µg/ml, such as at least 500 µg/ml, for example at least 750 µg/ml, such as at least 1000 µg/ml, for example at least 1300 µg/ml total protein or protein containing material. Hence, the composition may for example comprise in then range from 300 to 3000 µg/ml, such as in the range of 500 to 3000 µg/ml, for example in then range from 750 to 3000 µg/ml, such as in the range of 1000 to 3000 µg/ml, for example in then range from 1300 to 3000 µg/ml, such as in the range of 1000 to 2000 µg/ml total protein or protein containing material.

[0067] The other proteins may be any suitable proteins. In one embodiment the pharmaceutical composition may apart from MBL comprise one or more proteins naturally associated with MBL.

[0068] Hence, in one embodiment the present invention relates to a pharmaceutical composition, wherein the total protein apart from MBL furthermore comprises one or more MBL associated serine protease (MASP) or variants thereof. For example MASP and MASP variants may be selected from the group consisting of MASP-1 of SEQ ID NO:2, MASP-2 of SEQ ID NO:3, MASP-3 of SEQ ID NO:4, fragments thereof and functional homologues thereof. Functional homologues of MASP proteins are described herein below.

#### Pharmaceutical composition

[0069] In one aspect the present invention relates to a pharmaceutical composition. The pharmaceutical composition may be formulated in a number of different manners, depending on the purpose for the particular pharmaceutical composition.

For example the pharmaceutical composition may be formulated in a manner so it is useful for a particular administration form. Preferred administration forms are described herein below.

[0070] In one embodiment the pharmaceutical composition is formulated so it is a liquid. For example the composition may be a protein solution or the composition may be a protein suspension. Said liquid may be suitable for parenteral administration, for example for injection or infusion.

[0071] In preferred embodiments of the invention the pharmaceutical composition is suitable for bolus injection, which requires that the concentration of MBL is sufficiently high so that a suitable MBL dosage (see preferred MBL dosages herein below) may be contained in a volume, which may be administered by bolus injection. Pharmaceutical compositions for bolus injection preferably comprises at least 500 µg/ml, more preferably at least 750 µg/ml, even more preferably at least 1000 µg/ml, for example at least 1300 µg/ml MBL and/or MBL variants.

[0072] The liquid may be any useful liquid, however it is frequently preferred that the liquid is an aqueous liquid. For many purposes, in particular when the liquid should be used for parenteral administration, it is furthermore preferred that the liquid is sterile. Sterility may be conferred by any conventional method, for example filtration, irradiation or heating.

[0073] In addition it is preferred that pharmaceutical compositions for parenteral administration have been subjected to a virus reduction step, i.e. virus filtration and/or acidic treatment. The purpose of virus filtration is a reduction of any virus contaminants. The filters used therefore are in general made of several hollow fibre membranes, where virus and other contaminants are separated from the protein solution by size exclusion. The principle behind separation is the differences between MBL and virus permeability through the hollow fibres. The difference in permeability of protein and virus is greatly increased, when the solution pass through multiple layers, hence preferably the filters used comprises several layers. Virus filtration may be performed by any conventional method known to the person skilled for example as described in example 1 herein below.

In one embodiment of the present invention the liquid may comprise one or more lipophile vehicles, for example one or more lipophile vehicle suitable for controlled release of MBL and/or MBL variants.

[0074] The liquid may furthermore contain any suitable pharmaceutically acceptable additives. Preferred pharmaceutically acceptable additives are described herein below.

[0075] For storage purposes the protein solution or protein suspension may be stored at any desirable temperature, for example from -100°C to 0°C, such as from 0°C to 4°C, for example from 4°C to 10°C, such as from 10°C to 15°C, for example from 15°C to 25°C, such as from 15°C to 35°C.



[0076] Accordingly, in one embodiment of the invention the solution or suspension may be frozen. For most purposes the solution or suspension is only frozen for storage purposes and must be defrozen prior to use.

[0077] The pharmaceutical composition may be packaged in single dosage units, which may be more convenient for the user. Hence, pharmaceutical compositions for bolus injections may be packages in dosage units of for example at the most 10 ml, preferably at the most 8 ml, more preferably at the most 6 ml, such as at the most 5 ml, for example at the most 4 ml, such as at the most 3 ml, for example around 2.2 ml.

[0078] The pharmaceutical composition may be packaged in any suitable container. In one example a single dosage of the pharmaceutical composition may be packaged in injection syringes.

[0079] In another embodiment of the present invention the pharmaceutical composition is a dry composition. The dry composition may be used as such, but for most purposes the composition is a dry composition for storage only. Prior to use the dry composition may be dissolved or suspended in a suitable liquid composition, for example sterile water.

[0080] Hence, the pharmaceutical composition may be a dry composition, comprising freeze-dried protein. Preferably, said dry composition is capable of being reconstituted into a solution or suspension.

[0081] The protein may be freeze-dried using any conventional method known to the person skilled in the art. For freeze drying it may be necessary to add one or more cryoprotecting agents. Examples of preferred cryoprotecting agents are given herein below.

[0082] In one embodiment of the invention the pharmaceutical composition comprises one or more further active compounds in addition to MBL and/or MBL variants. Such a pharmaceutical composition may also be provided in the form of a kit-of-parts.

#### Administration

[0083] The pharmaceutical composition may be prepared so it is suitable for one or more particular administration methods. Furthermore, the method of treatment described herein may involve different administration methods.

[0084] In general any administration method, wherein MBL may be administered to an individual in a manner so that active MBL may reach the site of disease may be employed with the present invention.

[0085] For example, the pharmaceutical compositions of the invention may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The compounds may also be administered by inhalation, that is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

[0086] Accordingly, In one embodiment of the present invention the administration form may be selected from the group consisting of injection, infusion, nasal administration, transdermal administration, pulmonary administration, targeting and iontophoretic administration.

In general, for injection and infusion the pharmaceutical composition should be a sterile liquid.

[0087] Injection may be injection to any preferred site, for example injection may be selected from the group consisting of intravenous, subcutaneous, intraarterial, intramuscular and intraperitoneal injection. Infusion is generally intravenous infusion.

[0088] In one preferred embodiment of the present invention the administration form is a bolus injection. For such an embodiment it is preferred that the pharmaceutical composition comprises a high concentration of MBL, so sufficient MBL may be administered. It is an advantage of the present invention that the pharmaceutical compositions provided comprises high MBL concentrations and accordingly, allows sufficient MBL to be administered as bolus injection.

[0089] Furthermore, the route of administration may be topical administration to a mucosal membrane. The mucosal membrane to which the pharmaceutical preparation of the invention is administered may be any mucosal membrane of the mammal to which the biologically active substance is to be given, e.g. in the nose, vagina, eye, mouth, genital tract, lungs, gastrointestinal tract, or rectum.

[0090] The pharmaceutical compositions according to the present invention may be administered once or more than once, for example they may be administered in the range of 2 to 5 times, such as 5 to 10 times, for example 10 to 20 times, such as 20 to 50 times, for example 50 to 100 times, such as more than 100 times.

[0091] The dosage of MBL to be administered depends on the individual to be treated as well as on the clinical condition. In general, in the range of 0.1 mg to 10 mg, such as in the range of 0.5 mg to 5 mg, for example around 1 mg MBL per 10 kg bodymass may be administered. In one embodiment of the invention the dosage is in the range of 2 to 12 mg, such as in the range of 3 to 10 mg, for example in the range of 4 to 9 mg, such as in the range of 5 to 8 mg, for example in the range of 6 to 7 mg, such as around 6.6 mg MBL per administration for an adult human being.

[0092] The pharmaceutical compositions according to the invention may be administered with at least one other active compound. The compounds may be administered simultaneously, either as separate formulations or combined in a unit dosage form, or administered sequentially.

**[0093]** In one embodiment of the present invention the pharmaceutical composition may be administered as part of a diagnostic procedure. For example, diagnosis may be determination of the level of one or more compounds in an individual. For diagnostic purposes it may be required to administer pharmaceutical compositions comprising high levels of MBL to said individual, for example prior to said determination.

#### Stability of pharmaceutical compositions

**[0094]** Preferably, the pharmaceutical formulations according to the present invention are stable, i.e. they may be long-term stored without massive loss of activity and/or without major changes in size distribution. In particular, it is preferred that MBL and/or MBL variants comprised within the pharmaceutical compositions are stable, and more preferably that MBL and/or MBL variants are stable during long-term storage.

**[0095]** By the term "stable" is meant, that MBL and/or MBL variants comprised within the pharmaceutical compositions preferably does maintain at least part of the initial activity during long-term storage. Hence, preferably MBL and/or MBL variants comprises at least 20%, more preferably at least 30%, even more preferably at least 40%, even more preferably at least 50%, even more preferably at least 60%, even more preferably at least 70%, even more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99%, for example essentially all of the initial activity after long-term storage.

**[0096]** Alternatively, it is preferred that a long-term stored MBL and/or MBL variant sample comprises at least 20%, such as at least 30%, for example at least 40%, such as at least 50%, for example at least 60%, such as at least 70%, for example at least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99%, for example essentially all of the activity of an MBL sample stored at -80°C.

**[0097]** The activity of MBL may for example be determined by its capacity to activate or inactivate the complement system. When C4 is cleaved by MBL/MASP an active thiol ester is exposed and C4 becomes covalently attached to nearby nucleophilic groups. When an assay is performed in a coated plastic well, a substantial part of the C4b will become attached to the coated plastic well and may be detected by anti-C4 antibody. A quantitative TRIFMA for MBL activity may be performed by 1) coating microtitre wells with 1 g mannan in 100 l buffer; 2) blocking with Tween-20; 3) applying MBL complexes at a predetermined amount, applying test samples, 5) applying purified complement factor C4 at 5 g/ml; 6) incubate for one hour at 37°C; 7) applying Eu-labelled anti-C4 antibody; 8) applying enhancement solution; and 9) reading the Eu by time resolved fluorometry. (Estimation by ELISA may be carried out similarly, e.g. by applying biotin-labelled anti-C4 in step 7; 8) apply alkaline phosphatase-labelled avidin; 9) apply substrate; and 10) read the colour intensity). Between each step the plate was incubated at room temperature and washed, except between step 8 and 9. A calibration curve can be constructed using dilutions of MBL with known activity. The assay is preferably carried out at conditions which preclude activation of C4 by the classical or alternative complement activation pathways. The activation of C4 was completely inhibited by the serine protease inhibitor benzamidine. Activation of the classical pathway is effectively eliminated by carrying out step 3) in the presence of sufficiently high ionic strength (0.7 to 2.0 M NaCl; preferably about 1.0 M NaCl) which does not interfere with the MBL complex but completely destroys the C1qrs complex; activation of the alternative pathway is effectively precluded by assaying at dilution as described above.

It is however preferred that the activity of MBL is determined according to the C4 activation assay described in example 9 herein below.

**[0098]** Preferably, stable MBL and/or MBL variants are not broken down or only broken down in a limited amount during long-term storage, i.e. preferably stable MBL and/or MBL variants are not broken down or only broken down in a limited amount by either physically and/or chemical break down during storage. Accordingly, preferably the majority of polypeptides comprised within the MBL and/or MBL variants are of the same length (i.e. are containing the same amount of amino acids) after storage as before storage. The lengths of MBL and/or MBL variants and the amino acid content as well as MBL protein concentration may be determined by a number of different methods, for example by amino acid analysis, anion exchange chromatography, by coomassie staining on reduced MBL or by non-reduced MBL western blots, by mass spectrometry or by size exclusion chromatography.

**[0099]** Hence, preferably at least 10%, such as at least 20%, such as at least 30%, for example at least 40%, such as at least 50%, for example at least 60%, such as at least 70%, for example at least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99% of the MBL/MBL variant polypeptides are at least 50%, for example at least 60%, such as at least 70%, for example at least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99%, for example around 100% of the initial length.

**[0100]** Furthermore, it is preferred that at least 50%, for example at least 60%, such as at least 70%, for example at

least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99% of the MBL/MBL variant polypeptides show no changes in the polypeptide chain after storage when investigated by mass spectrometry.

[0101] In addition it is preferred that at least 50%, for example at least 60%, such as at least 70%, for example at least 80%, such as at least 85%, for example at least 90%, such as at least 95%, for example at least 96%, such as at least 97%, for example at least 98%, such as at least 99% of the stored MBL/MBL variant polypeptides eluates similar to initial MBL/MBL variants or MBL/MBL variants stored at - 80°C after size exclusion chromatography. Size exclusion chromatography may for example be performed as described in example 10 herein below.

[0102] By the term "initial length" is meant the length of MBL/MBL variant polypeptides before storage or alternatively the length of MBL/MBL variant polypeptides stored at - 80°C, which in one preferred embodiment of the invention corresponds to full length MBL, for example the full length of the polypeptide as identified by SEQ ID NO.1. In general the length of MBL/MBL variant may be determined by determining the molecular weight of MBL/MBL variant polypeptides as measured in Daltons.

[0103] By the term long-term storage is meant storage for in the range of 1 to 4 weeks, such as 1 to 3 months, such as 3 to 6 months, such as 6 to 12 months, for example 1 to 2 years, such as 2 to 3 years, for example 3 to 5 years, such as 5 to 7 years, for example 7 to 10 years, such as more than 10 years. Preferably, long-term storage is in the range of 1 to 2 years, more preferably in the range of 2 to 5 years.

[0104] The stability of MBL and/or MBL variants is dependent on the storage conditions. Preferably, MBL and/or MBL variants are stable when stored at a temperature for example in the range of -80°C to -20°C, such as -25°C to -15°C, for example -15°C to 0°C, such as 0°C to 4°C, for example 2°C to 10°C, such as 5°C to 15°C, for example -10°C to 20°C, such as 15°C to 35°C, for example 15°C to 30°C, such as 15°C to 25°C. In one preferred embodiment of the invention MBL and/or MBL variants may be stored at temperatures around 4°C, in another preferred embodiment of the invention MBL and/or MBL variants may be stored at temperatures around - 20°C, in yet another preferred embodiment of the invention MBL and/or MBL variants may be stored at temperatures around 37°C.

[0105] The stability of MBL and/or MBL variants may furthermore be dependent on the nature of the pharmaceutical composition. For example, the stability of MBL and/or MBL variants may be different when the pharmaceutical composition is a solution, a suspension or dry matter.

#### Functional homologues

[0106] Functional homologues of polypeptides according to the present invention is meant to comprise any polypeptide sequence which is capable of performing essentially the same function or partially the same function as a polypeptide of a specific predetermined sequence.

Accordingly, functional homologues according to the present invention comprise polypeptides with an amino acid sequence, which are sharing at least some homology with a predetermined polypeptide sequences as outlined herein above.

[0107] Preferably, functional homologues of human MBL comprises polypeptides which share at least some homology with the polypeptides as identified by SEQ ID NO 1. Preferably, functional homologues of human MASP-1 comprises polypeptides which share at least some homology with the polypeptides as identified by SEQ ID NO 2. Preferably, functional homologues of human MASP-2 comprises polypeptides which share at least some homology with the polypeptides as identified by SEQ ID NO 3. Preferably, functional homologues of human MASP-3 comprises polypeptides which share at least some homology with the polypeptides as identified by SEQ ID NO 4.

[0108] For example such polypeptides are at least about 40 percent, such as at least about 50 percent homologous, for example at least about 60 percent homologous, such as at least about 70 percent homologous, for example at least about 75 percent homologous, such as at least about 80 percent homologous, for example at least about 85 percent homologous, such as at least about 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous with the predetermined polypeptide sequences as outlined herein above.

[0109] Homology may preferably be calculated by any suitable algorithm or by computerised implementations of such algorithms for example CLUSTAL in the PC/Gene program by Intelligenetics or GAP, BESTFIT, BLAST, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG). The homology between amino acid sequences may furthermore be calculated with the aid of well known matrices such as for example any one of BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, and BLOSUM 90.

[0110] Functional homologues may comprise an amino acid sequence that comprises at least one substitution of one amino acid for any other amino acid. For example such a substitution may be a conservative amino acid substitution or it may be a non-conservative substitution.

**[0111]** A conservative amino acid substitution is a substitution of one amino acid within a predetermined group of amino acids for another amino acid within the same group, wherein the amino acids within a predetermined groups exhibit similar or substantially similar characteristics. Within the meaning of the term "conservative amino acid substitution" as applied herein, one amino acid may be substituted for another within groups of amino acids characterised by having

i) polar side chains (Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, Tyr, and Cys,)

ii) non-polar side chains (Gly, Ala, Val, Leu, Ile, Phe, Trp, Pro, and Met)

iii) aliphatic side chains (Gly, Ala, Val, Leu, Ile)

iv) cyclic side chains (Phe, Tyr, Trp, His, Pro)

v) aromatic side chains (Phe, Tyr, Trp)

vi) acidic side chains (Asp, Glu)

vii) basic side chains (Lys, Arg, His)

viii) amide side chains (Asn, Gln)

ix) hydroxy side chains (Ser, Thr)

x) sulphur-containing side chains (Cys, Met), and

xi) amino acids being monoamino-dicarboxylic acids or monoamino-monocarboxylic-monoamidocarboxylic acids (Asp, Glu, Asn, Gln).

**[0112]** Functional homologues according to the present invention may comprise more than one such substitution, such as e.g. two amino acid substitutions, for example three or four amino acid substitutions, such as five or six amino acid substitutions, for example seven or eight amino acid substitutions, such as from 10 to 15 amino acid substitutions, for example from 15 to 25 amino acid substitution, such as from 25 to 30 amino acid substitutions, for example from 30 to 40 amino acid substitution, such as from 40 to 50 amino acid substitutions, for example from 50 to 75 amino acid substitution, such as from 75 to 100 amino acid substitutions, for example more than 100 amino acid substitutions.

**[0113]** The addition or deletion of an amino acid may be an addition or deletion of from 2 to 5 amino acids, such as from 5 to 10 amino acids, for example from 10 to 20 amino acids, such as from 20 to 50 amino acids. However, additions or deletions of more than 50 amino acids, such as additions from 50 to 200 amino acids, are also comprised within the present invention.

**[0114]** The polypeptides according to the present invention, including any variants and functional homologues thereof, may in one embodiment comprise more than 5 amino acid residues, such as more than 10 amino acid residues, for example more than 20 amino acid residues, such as more than 25 amino acid residues, for example more than 50 amino acid residues, such as more than 75 amino acid residues, for example more than 100 amino acid residues, such as more than 150 amino acid residues, for example more than 200 amino acid residues.

**[0115]** Additional factors may be taken into consideration when determining functional homologues according to the meaning used herein. For example functional homologues may be capable of associating with antisera which are specific for the polypeptides according to the present invention.

**[0116]** Peptides with N-terminal alkylations and C-terminal esterifications are also encompassed within the present invention. Functional homologues also comprise post-translationally modified polypeptides, for example glycosylated polypeptides.

#### Pharmaceutically acceptable additives

**[0117]** The pharmaceutical compositions containing MBL or MBL variants may be prepared by any conventional technique, e.g. as described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa.

**[0118]** The pharmaceutical acceptable additives may be any conventionally used pharmaceutical acceptable additive, which should be selected according to the specific formulation, intended administration route etc. For example

the pharmaceutical acceptable additives may be any of the additives mentioned in Nema et al, 1997. Furthermore, the pharmaceutical acceptable additive may be any accepted additive from FDA's "inactive ingredients list", which for example is available on the internet address <http://www.fda.gov/cder/drug/iig/default.htm>.

[0119] In some embodiments of the present invention it is desirable that the pharmaceutical composition comprises an isotonic agent. In particular when the pharmaceutical composition is prepared for administration by injection or infusion it is often desirable that an isotonic agent is added.

[0120] Accordingly, the composition may comprise at least one pharmaceutically acceptable additive which is an isotonic agent.

[0121] The pharmaceutical composition may be isotonic, hypotonic or hypertonic. However it is often preferred that a pharmaceutical composition for infusion or injection is essentially isotonic, when it is administered. Hence, for storage the pharmaceutical composition may preferably be isotonic or hypertonic. If the pharmaceutical composition is hypertonic for storage, it may be diluted to become an isotonic solution prior to administration.

[0122] The isotonic agent may be a ionic isotonic agent such as a salt or a non-ionic isotonic agent such as a carbohydrate.

[0123] Examples of ionic isotonic agents include but are not limited to NaCl, CaCl<sub>2</sub>, KCl and MgCl<sub>2</sub>. Examples of non-ionic isotonic agents include but are not limited to mannitol and glycerol.

[0124] It is also contained within the present invention that at least one pharmaceutically acceptable additive is a buffer. For some purposes, for example, when the pharmaceutical composition is meant for infusion or injection, it is often desirable that the composition comprises a buffer, which is capable of buffering a solution to a pH in the range of 4 to 10, such as 5 to 9, for example 6 to 8.

[0125] However, in other embodiments of the invention the pharmaceutical composition may comprise no buffer at all or only micromolar amounts of buffer.

[0126] The buffer may for example be selected from the group consisting of TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine buffer. Hence, the buffer may be K<sub>2</sub>HPO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub> or sodium citrate.

[0127] TRIS buffer is known under various other names for example tromethamine including tromethamine USP, THAM, Trizma, Trisamine, Tris amino and trometamol. The designation TRIS covers all the aforementioned designations.

[0128] The buffer may furthermore for example be selected from USP compatible buffers for parenteral use, in particular, when the pharmaceutical formulation is for parenteral use. For example the buffer may be selected from the group consisting of monobasic acids such as acetic, benzoic, gluconic, glyceric and lactic, dibasic acids such as aconitic, adipic, ascorbic, carbonic, glutamic, malic, succinic and tartaric, polybasic acids such as citric and phosphoric and bases such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS.

[0129] In preferred embodiments of the present invention, the pharmaceutical compositions according to the present invention do not contain stabilisers, in particular it is preferred that the pharmaceutical compositions do not contain protein containing stabilisers, even more preferably the pharmaceutical compositions do not contain protein containing stabilisers and detergents. Pharmaceutical formulations wherein the active ingredient is a protein or a peptide in general comprise one or more stabilisers. Said stabilisers are frequently one or more proteins or one or more detergents. It is frequently desirable to reduce the amount of additives in a pharmaceutical composition. Hence, preferred pharmaceutical compositions according to the present invention do not contain any protein and/or detergent.

[0130] However, in other embodiments of the invention pharmaceutical compositions, may comprise at least one pharmaceutically acceptable additive which is a stabiliser. The stabiliser may for example be a detergent, an amino acid, a fatty acid, a polymer, a polyhydric alcohol, a metal ion, a reducing agent, a chelating agent or an antioxidant, however any other suitable stabiliser may also be used with the present invention.

[0131] For example the stabiliser may be selected from the group consisting of poloxamers, Tween-20, Tween-40, Tween-60, Tween-80, Brij, metal ions, amino acids, polyethylene glycol, Triton, EDTA and ascorbic acid.

[0132] Furthermore, the stabiliser may be selected from the group consisting of amino acids such as glycine, alanine, arginine, leucine, glutamic acid and aspartic acid, surfactants such as polysorbate 20, polysorbate 80 and poloxamer 407, fatty acids such as phosphatidyl choline ethanolamine and acetyltryptophanate, polymers such as polyethylene glycol and polyvinylpyrrolidone, polyhydric alcohol such as sorbitol, mannitol, glycerin, sucrose, glucose, propylene glycol, ethylene glycol, lactose and trehalose, antioxidants such as ascorbic acid, cysteine HCL, thioglycerol, thioglycolic acid, thiosorbitol and glutathione, reducing agents such as several thiols, chelating agents such as EDTA salts, glutamic acid and aspartic acid and metal ions such as Ca<sup>++</sup>, Ni<sup>++</sup>, Mg<sup>++</sup> and Mn<sup>++</sup>.

[0133] Other examples of antioxidants and reducing agents useful with the present invention includes acetone sodium bisulfite, ascorbate, bisulfite sodium, butylated hydroxy anisole, butylated hydroxy toluene, cystein/cysteinate HCL, dithionite sodium, gentisic acid, gentisic acid ethanolamine, glutamate monosodium, formaldehyde sulfoxylate sodium, metabisulfite potassium, metabisulfite sodium, monothioglycerol, propyl gallate, sulfite sodium and thioglycolate sodium.

[0134] The pharmaceutical composition according to the invention may also comprise one or more cryoprotectant agents. In particular, when the composition comprises freeze-dried protein or the composition should be stored frozen it may be desirable to add a cryoprotecting agent to the pharmaceutical composition.

[0135] The cryoprotectant agent may be any useful cryoprotectant agent, for example the cryoprotectant agent may be selected from the group consisting of dextran, glycerin, polyethyleneglycol, sucrose, trehalose and mannitol.

[0136] Accordingly, the pharmaceutically acceptable additives may comprise one or more selected from the group consisting of isotonic salt, hypertonic salt, buffer and stabilisers. Furthermore, the pharmaceutically acceptable additives may comprise one or more selected from the group consisting of isotonic agents, buffer, stabilisers and cryoprotectant agents. For example, the pharmaceutically acceptable additives comprise glucosemonohydrate, glycine, NaCl and polyethyleneglycol 3350.

[0137] In another aspect the invention relates to an MBL composition comprising one or more cations.

[0138] Surprisingly, the addition of cations to an MBL solution may result in an increased stability of MBL and/or MBL variants. Preferably, the compositions comprises in the range of 0.01 mM to 1000 mM, more preferably in the range of 0.05 mM to 500 mM, even more preferably in the range of 0.1 mM to 100 mM, yet more preferably in the range of 0.2 mM to 50 mM, even more preferably in the range of 0.3 mM to 25 mM, yet more preferably in the range of 0.5 mM to 10 mM, such as in the range of 0.5 mM to 5 mM, for example in the range of 0.5 mM to 2 mM, such as around 1 mM divalent cation. The cation is preferably a divalent cation, and accordingly the invention relates to an MBL composition comprising one or more divalent cations.

[0139] The divalent cation is preferably a metal divalent cation. For example the divalent cation may be selected from the group consisting of  $\text{Ca}^{++}$ ,  $\text{Ni}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{Mn}^{++}$ . Preferably, however the divalent cation is  $\text{Ca}^{++}$ .  $\text{Ca}^{++}$  may be added to the solution as any suitable salt, for example as  $\text{CaCl}_2$ .

[0140] In a preferred embodiment the MBL composition is as described above with respect to MBL content.

#### Clinical condition

[0141] A number of clinical conditions may be treated using the pharmaceutical compositions according to the present invention. In particular however the following conditions may be treated:

1. Infections
2. MBL deficiency
3. Immunocompromised conditions

[0142] Infections may for example be an infection by bacteria, fungi, viruses, parasites. For example infection by one or more bacteria selected from the group consisting of *Achromobacter xylosoxidans*, *Acinetobacter calcoaceticus*, preferably *A. anitratus*, *A. haemolyticus*, *A. alcaligenes*, and *A. lwoffii*, *Actinomyces israelii*, *Aeromonas hydrophilia*, *Alcaligenes* species, preferably *A. faecalis*, *A. odorans* and *A. denitrificans*, *Arizona hinshawii*, *Bacillus anthracis*, *Bacillus cereus*, *Bacteroides fragilis*, *Bacteroides melaninogenicus*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Borrelia recurrentis*, *Brucella* species, preferably *B. abortus*, *B. suis*, *B. melitensis* and *B. canis*, *Calymmatobacterium granulomatis*, *Campylobacter fetus* ssp. *intestinalis*, *Campylobacter fetus* ssp. *jejuni*, *Chlamydia* species, preferably *C. psittaci* and *C. trachomatis*, *Chromobacterium violaceum*, *Citrobacter* species, preferably *C. freundii* and *C. diversus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Corynebacterium*, preferably *C. ulcerans*, *C. haemolyticum* and *C. pseudotuberculosis*, *Coxiella burnetii*, *Edwardsiella tarda*, *Eikenella corrodens*, *Enterobacter*, preferably *E. cloacae*, *E. aerogenes*, *E. hafniae* (also named *Hafnia alvei*) and *E. agglomerans*, *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Flavobacterium meningosepticum*, *Francisella tularensis*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Helicobacter* species, *Klebsiella* species, preferably *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*, *Legionella* species, *Leptospira interrogans*, *Listeria monocytogenes*, *Moraxella* species, preferably *M. lacunata* and *M. osloensis*, *Mycobacterium bovis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma* species, preferably *M. pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia* species, preferably *N. asteroides* and *N. brasiliensis*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Peptococcus magnus*, *Plesiomonas shigelloides*, *Pneumococci*, *Proteus* species, preferably *P. mirabilis*, *P. vulgaris*, *P. rettgeri* and *P. morganii* (also named *Providencia rettgeri* and *Morganella morganii* respectively), *Providencia* species, preferably *P. alcalifaciens*, *P. stuartii* and *P. rettgeri* (also named *Proteus rettgeri*), *Pseudomonas aeruginosa*, *Pseudomonas mallei*, *Pseudomonas pseudomallei*, *Rickettsia*, *Rochalimma henselae*, *Salmonella* species, preferably *S. enteridis*, *S. typhi* and *S. derby*, and most preferably *Salmonella* species of the type *Salmonella* DT104, *Serratia* species, preferably *S. marcescens*, *Shigella dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*, *Spirillum minor*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptobacillus moniliformis*, *Streptococcus*, preferably *S. faecalis*, *S. faecium* and *S. durans*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema carateum*, *Treponema pallidum*,

Treponema pertenu, preferably T. pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica, and Yersinia pestis.

[0143] Immuno compromised conditions includes any functional deficiency of one or more components of the immune system, for example one or more components of the cellular immune system, such as for example immune cells or one or more components of the humoral immune system. The deficiency may be caused by lack of the component or by the presence of reduced amounts of the component or may be due to functional alterations of the component or parts of the component.

[0144] In one example the immunocompromised condition may be MASP deficiency.

#### Individual in need of MBL treatment

[0145] The individual in need of MBL treatment or a medicament comprising MBL according to the invention may be any individual, for example it may be an individual suffering from a clinical condition or an individual in risk of acquiring a clinical condition. Preferably, the individual is a human being.

[0146] In a preferred embodiment of the present invention, the individual is suffering from one of the clinical conditions described herein above. In another preferred embodiment the individual is at increased risk of acquiring a clinical condition as described herein above.

[0147] For example the individual may have increased risk of acquiring an infection. The increased risk may have several different reasons, however frequently the increased risk will be associated with a functional deficiency in one or more components of the immune system. The functional deficiency of the immune system may be a deficiency in the cellular immune system and/or the humoral immune system.

[0148] Components of the cellular immune systems involves for example neutrophils, T-cells, B-cells, basophiles and phagocytes. The functional deficiency may be caused by a reduced number of one or more of said cells or by malfunction of one or more of said cells.

[0149] Components of the humoral immune systems includes for example the complement pathway and the lectin complement pathway. Accordingly, in one embodiment of the invention, the individual is an individual with a functional deficiency of MBL. For example the individual may have a subnormal serum level of MBL.

[0150] A subnormal MBL serum level refers to a concentration of MBL in serum, which is lower than the normal serum concentration of MBL. For example the MBL serum level may be <2000 ng/ml, such as <1500 ng/ml, for example <1000 ng/ml, such as <900 ng/ml, for example <800 ng/ml, such as <700 ng/ml, for example <600 ng/ml, such as <500 ng/ml, for example <400 ng/ml, such as <300 ng/ml, for example <200 ng/ml, such as <100 ng/ml.

#### Examples

[0151] The following examples illustrate selected embodiments of the present invention and should not be regarded as limiting for the invention.

#### Example 1

[0152]

MBL formulation	
Content	Concentration
MBL	1 mg/ml
Tris-buffer	10 mM, pH 7,4
NaCl	140 mM

[0153] MBL is recombinant human MBL prepared and purified as described in PCT application WO00/70043 Briefly, the genomic human MBL gene was amplified by PCR and inserted into a pREP9 vector (cat.no. V009-50, Invitrogen). HEK 293EBNA cells were transfected with the construct and culture medium from the MBL/pREP9 transfected cells, was passed over a column of Fractogel TSK HW-75 beads (cat.no. 14985, Merck KGaA, Darmstadt, Germany), which had been coupled to mannose and been pre-washed in 15 mL of 0.1 M glycine (pH 3.0) and equilibrated in TBS/0.05% (v/v) Tween-20, 2 mM CaCl<sub>2</sub>. The column was washed in 75 mL of TBS, 2 mM CaCl<sub>2</sub>.

[0154] Recombinant MBL is eluted in a buffer containing 10 mM Tris-buffer and 140 mM NaCl in the last purification step. Subsequently, the formulation is virus filtered over a Planova filter consisting of 150 layers, which leads to high virus retention (7 logs).

[0155] Several Planova filters exist and the filters of size 75N and 35N are selected for virus filtration of MBL solution. The 75N and 35N were selected after a study of the MBL recovery using Planova filters with different pore size. Filters 15N and 20N are commercial available but the pore size is too small for filtrating MBL. The 75N filter will only filter larger virus like HIV and operate more as a prefilter than a virus removal filter. The Planova 35N will remove virus larger than 35 nm size, which include reovirus but not smaller vira like polio- and parvovirus. For smaller virus, an acidic treatment step during MBL purification is assumed to be effective for virus inactivation.

[0156] First, the MBL solution is passed through the 75N filter and secondly the filtrate is thereafter passed through the 35N filter. Both filtrations are carried out as dead end filtration. For laboratory scale a 0.01 m<sup>2</sup> filter is used. In pilot production scale, a filter of 0.3 m<sup>2</sup> is used.

[0157] Finally, the MBL solution is diluted in 10 mM Tris-buffer and 140 mM NaCl to a final concentration of 1 mg/ml, sterile filtered over a 0.22 µm filter and aliquoted into 2.2 ml aliquots.

[0158] This formulation is suitable for parenteral administration. In particular, 1 aliquot may be administered to a human child (depending on age) and 3 aliquots may be administered to a human adult.

## Example 2

[0159]

MBL formulation	
Content	In 1 ml of the formulation:
MBL	1 mg
Tris-buffer	1.2 mg
NaCl	5.8 mg
Mannitol	7.3 mg

[0160] MBL is recombinant human MBL prepared and purified as described in PCT application WO00/70043. Recombinant MBL is eluted in a buffer containing 1.2 mg/ml Tris-buffer and 5.8 mg/ml NaCl in the last purification step. Subsequently, 7.3 mg/ml mannitol is added to the MBL solution. The formulation is sterile filtered prior to use.

[0161] This formulation is suitable for parenteral administration.

## Example 3

[0162]

MBL formulation	
Content	In 1 ml of the formulation:
MBL	1 mg
Tris-buffer	1.2 mg
Nacl	5.8 mg
Mannitol	7.3 mg
Tween-80	0.1 mg

[0163] MBL is recombinant human MBL prepared and purified as described in PCT application WO00/70043. Recombinant MBL is eluted in a buffer containing 1.2 mg/ml Tris-buffer and 5.8 mg/ml Nacl in the last purification step. Subsequently, 7.3 mg/ml mannitol and 0.1 mg Tween-80 is added to the MBL solution. The formulation is steril filtered and virus filtered (see example 1) prior to use.

[0164] This formulation is suitable for parenteral administration.



Example 4

MBL formulation

[0165]

10 mg/ml MBL  
10 mM tris, pH = 7.4.  
140 mM NaCl

[0166] Recombinant MBL is prepared as described in example 1. MBL is then concentrated using a filtration device exposing the protein to 3500 x G. The formulation is sterile filtered and virus filtered (see example 1) prior to use.

[0167] The formulation is suitable for parenteral administration.

Example 5:

[0168]

MBL: 1 mg/ml  
Trisbuffer: 10 mM  
NaCl: 140 mM  
CaCl<sub>2</sub>: 0.1 mM

[0169] Recombinant MBL is prepared as described in example 1. CaCl<sub>2</sub> is added to a final concentration of 1 mM. The formulation is sterile filtered and virus filtered (see example 1) prior to use.

[0170] The formulation is suitable for parenteral administration.

Example 6:

[0171]

MBL: 5 mg/ml  
Trisbuffer: 10 mM  
NaCl: 140 mM  
CaCl<sub>2</sub>: 1 mM

[0172] Recombinant MBL is prepared as described in example 1. MBL is then concentrated using a filtration device exposing the protein to 3500 x G. CaCl<sub>2</sub> is added to a final concentration of 1 mM. The formulation is sterile filtered and virus filtered (see example 1) prior to use.

[0173] The formulation is suitable for parenteral administration.

Example 7

[0174]

MBL: 10 mg/ml  
Trisbuffer: 10 mM  
NaCl: 140 mM  
CaCl<sub>2</sub>: 1 mM

[0175] Recombinant MBL is prepared as described in example 1. MBL is then concentrated using a filtration device exposing the protein to 3500 x G. CaCl<sub>2</sub> is added to a final concentration of 1 mM. The formulation is sterile filtered and virus filtered (see example 1) prior to use.

[0176] The formulation is suitable for parenteral administration.

Example 8Stability of pharmaceutical formulations comprising MBL

5 [0177] Recombinant MBL has been formulated as a liquid solution, and stability tested in storage material acceptable for liquid protein storage. A number of three different formulations with different concentrations of MBL were tested and one formulation was tested for a stabilizing effect of calcium ions.

10 [0178] A formulation on 1 mg/ml without any protein stabilizing excipients showed surprisingly high stability when stored frozen (-20 °C) and chilled (+4°C) for 12 months. No activity decrease was measured nor were changes in the polypeptide chain when investigated by mass spectrometry. No changes were observed in protein content by amino acid analysis or anion exchange chromatography. No degradation products were observed by coomassie staining on reduced MBL or by non-reduced MBL western blots. A small front peak, which probably is caused by aggregated MBL, corresponding to only approximately 1 % of total MBL content was observed by size exclusion chromatography. Results obtained for a higher MBL formulation (10 mg/ml and 5 mg/ml) indicate the possibility of aggregate formation caused by the stressful concentration step rather than by storage. Addition of calcium ions showed dramatically stabilizing effect with no changes in activity after 2 months of storage at +37 °C.

**INTRODUCTION**

20 [0179] Three pharmaceutical formulations comprising recombinant mannose binding lectin (rMBL) have been developed and filled into storage material acceptable for pharmaceutical use. Filling was done aseptically using only sterile vials, rubber stoppers and performed in a laminar air hood. Type of formulation, storage material and filling process therefore make the preparation highly representative of a MBL drug product for clinical use. These formulations were stability tested by storage at different temperatures and incubation time. Storage equipment were all temperature monitored.

25 The following MBL formulations were studied:

- PE0238: 1 mg/ml MBL in 10 mM tris, 140 mM NaCl and pH = 7.4.
- PE0305: 10 mg/ml MBL in 10 mM tris, 140 mM NaCl and pH = 7.4.
- 30 - PE0407: 5 mg/ml MBL in 10 mM tris, 140 mM NaCl, 1 mM CaCl<sub>2</sub> and pH = 7.4.

[0180] All references used in these studies are kept at -80 °C and accepted as stable when used.

**METHODS**The C4-activation assay

35 [0181] C4 activation assay was performed as described in example 9 herein below. The C4 activation assay measures the capability of MBL to bind to MASPs (mannose binding protein associated proteases), and upon binding to a carbohydrate target, activate the MASPs. The MBL/MASP complex subsequently cleaves C4 and initiates the lectin pathway of complement activation. C4 activation is therefore a measure of the biological potency of MBL. This assay is the major stability-indicating assay for MBL.

Size Exclusion chromatographic (SEC)

45 [0182] SEC is a chromatographic analysis performed on a Superose 6 column (Amersham Biosciences) as described in example 10 herein below; MBL and other components are separated by molecular volume, which for globular proteins is proportional to molecular mass.

50 [0183] The SEC analysis is used for verification of the high-mass structure of MBL. While high-mass oligomers elute early in a chromatogram, non-oligomers elute late in the chromatogram. Moreover, aggregates larger than oligomeric MBL can be detected, if present in the sample. Finally, the analysis might also be used for quantifying MBL content.

Mass Spectrometry (MALDI-MS)

55 [0184] Initially, the sample is reduced and purified according to a generic procedure to remove non-protein components and limit background signal. MALDI-MS is done to verify the mass profile of the MBL polypeptide chain and to identify potential degradation products of the polypeptide chain.

## RESULTS

## C4-activation assay

[0185] PE0238: The C4-activation assay indicate identical activity for the 1 mg/ml formulation, when stored 12 months at -20 and +4 degree C as for the reference. Results are shown in figure 1 as a six-point curve with EC50 values at 105% for +4 degree storage and 104% for -20 degree storage compared to the reference at 100%.

[0186] PE0305: The initial activity is measured to 94% relative to the standard reference for the 10 mg/ml formulation. After 2½ months the activity is decreased to around 80% for the frozen and chilled samples and to 36% for the +37 degree sample. After 5 months the activity is still around 80% for frozen and chilled samples, where a drop to 14.3% is measured for the +37 °C sample. Results are obtained by C4-activation stability curves and listed as EC50 values in table 1. Only changes of more than -15 % are considered as significant.

Table 1.

C4-activation on stability samples. The T = 0 analyse is a single estimation. Activity is calculated in EC50 % relative to a MBL standard. Measurements in triplicate are illustrated with standard variations.			
Type of storage	-20 °C	+4 °C	+37°C
T = 0 months	94% (N=1)	Same as -20 °C	Same as -20 °C
T=2½months	81% (N=1)	78% (N=1)	36% (N=1)
T = 5 months	81.7% ± 4.7 (N=3)	80.3% ± 7.6 (N=3)	14.3% ± 1.5 (N=3)

[0187] PE0407: The 5 mg/ml formulation was activity tested and surprisingly is only a very small activity decrease observed for the sample stored at +37 degree and compared to the frozen sample are no changes observed. Activities are measured to 91% for the frozen, 92 for the chilled sample and 94 for the sample at +37 degree C. A control without calcium was incubated at +37 degree and measured to 19% activity. Results are obtained by C4-activation curves and listed in table 2 as EC50 values.

Table 2:

C4-activation data on 5 mg/ml stability compared to the reference N105-11A, EC50= 100%.				
Type of storage	-20 °C	+4 °C	+37°C	Control 37°C
( T = 2 months	91%	92%	94%	19%

## SEC data.

[0188] PE0238: The 1 mg/ml formulation was analysed for detecting molecular mass changes. The reference elute at 20.4 minutes as a single peak. For chilled storage a small front peak is eluting at 15 minutes, the front peak corresponds to approximately 1 % of total MBL content. For frozen storage the peak is identical to the reference with no detection of front peak. Figure 2 illustrates the SEC chromatograms of the reference standard in top followed by 1 mg/ml stability sample kept at +4 degree and at -20 degree. A small front peak is detected in the sample kept chilled corresponding to 1% of the total MBL content.

[0189] PE305: SEC data from the 10 mg/ml formulation indicate generation of a front peak. The amount of MBL in the front peak is calculated by comparison to the total MBL content and illustrated in table 3 as front peak in % of total MBL. According to table 3 are front peaks detected in all samples corresponding to 3 to 5 % of total MBL for storage at frozen and chilled temperatures. At +37 degree 40% is observed after 2½ months and 56% is present in the front peak after 5 months of storage.

Table 3:

The amount of MBL present in the front peak in % of total MBL. All measurements are in duplicate.			
Type of storage	-20 °C	+4 °C	+37 °C
T = 2½ months	3.5% ± 1.6 (N=2)	5.3% ± 0.1 (N=2)	39.5% ± 3.0 (N=2)
T = 5 months	4.1% ± 1.6 (N=2)	3.4% ± 2.1 (N=2)	56.5% ± 16.1 (N=2)

[0190] PE0407: Front peaks are observed in the 5 mg/ml formulation with amounts between 1 and 3%. A control without calcium and incubated at +37 degree was the MBL content in front peak measured to 62%.

Table 4:

The amount of MBL present in the front peak in % of total MBL. All measurements are single estimations				
Type of storage	-20 °C	+4 °C	+37°C	Control 37°C
MM T = 2 months	2%(N=1)	1% (N=1)	3%(N=1)	62%(N=1)

#### MS data

[0191] PE238: Mass spectrometry analyses on reduced MBL indicate an intact MBL polypeptide chain during 12 months of storage at -20 and +4 degree, nor are changes observed after 6 months of storage at +37 degree. MS spectrograms are illustrated in figure 3.

MBL does not appear as a single peak due to different hydroxylation and glycosylation profiles, normally resulting in a total of seven peaks with the main peak around 25450 Da. Data do not indicate deglycosylation or polypeptide changes with storage time.

[0192] Figure 3 shows similar peaks for the reference and the 12 months samples indicate no changes in MBL polypeptide chain during storage.

#### DISCUSSION

[0193] The 1 mg/ml formulation is stable 12 months under frozen storage with no changes in C4-activation, no aggregates detected and no changes in polypeptide chain. Among other analyses performed are AIEC for content, non-reduced western blots for size distribution, coomassie staining for degradation products detection and amino acid analysis to verify content. None of these analyses indicate any change after 12 months storage at frozen and chilled temperatures. Approximately 99% of the total MBL elutes similar to reference MBL after 12 months storage at chilled temperature. C.

For the 10 mg/ml formulation a decrease in C4-activation is detected for all analysed samples to around 80 % after both 2½ and 5 months of storage at frozen and chilled temperature, indicating that once MBL has been concentrated to 10 mg/ml it is stable. The C4-activation value at time zero are measured to 94%. SEC analyses indicate an aggregated amount around 4% for both 2½ and 5 months of storage at frozen and chilled temperature. The amount of aggregate is constant with temperature and storage time indicating that aggregate might be present from study start in the concentrated formulations and caused by the concentration step rather than by storage. Any formulation higher than 1 mg/ml has been prepared by concentration using a filtration device exposing the protein to 3500 x G. A process step extremely stressful to any protein and a treatment generally known to cause protein aggregation.

[0194] The 5 mg/ml formulation has C4-activation results between 91 and 94% compared to the reference for storage at frozen, chilled and even at +37 degree. A control formulation without calcium placed at +37 degree is measured to 19% activity after two months of storage. SEC detects aggregation with amounts corresponding to 1 to 3 % of total MBL content for frozen and chilled samples and 62% for the control at +37 degree. Samples are not analysed by Maldi-MS.

Data from the three stability studies all indicate MBL as an exceptionally stable protein at frozen and chilled storage. The final conclusion is that a highly stable 1 mg/ml liquid MBL formulation has been developed for parenteral use without any stabilizing excipients. The addition of Calcium has a dramatically stabilizing effect on a concentrated formulation. A 5 mg/ml formulation stored for two months at +37 degree without any activity loss is exceptional for protein formulations.

#### Example 9

##### C4 activation assay

##### Coating FluoroNunc plates with Mannan.

[0195] FluoroNunc micro titer plates are coated with 100 µl/well of a solution containing 10 µg/ml of Mannan (Sigma-Aldrich, powder # M7504) dissolved in 50 mM NaCO<sub>3</sub> buffer pH 9.6 over night at 20 ± 4 °C in a humidified chamber. Plates are washed twice in TBS (Tris (10 mM), NaCl (150 mM) pH 7.4) before blocking the plates for 1 hour at 20 ± 4 °C with 200 µl per well of TBS buffer supplemented with 1 mg/ml HSA (Statens Serum Institute, Copenhagen). Each

well is washed three times with TBST buffer.

#### C4 activation assay

**[0196]** MASP is purified from an MBL deficient individual without anti Mannan antibodies. The rMBL reference standard originate from a purified rMBL reference material that has been pre-diluted into LISA buffer (Tris/Base (20 mM), NaCl (150 mM), CaCl<sub>2</sub> (10 mM), HSA 1 mg/ml, Triton X-100 (0.05%), PH 7.4) to a final concentration of 100 µg/ml and stored at -80 degrees Celsius.

**[0197]** The rMBL standard and the test samples is pre-diluted to 5 µg/ml in LISA buffer. The pre-diluted rMBL standard and the samples are mixed with the MASP in a 1:1 volume and incubated for 10 min at 37 degrees Celsius.

**[0198]** C4 complement factor is purified from fresh blood and stored frozen in C4, 1M NaCl, 20 mM Tris, 50 mM 6-amino-hexansyre, 5 mM EDTA, 0.02% W7v NaN<sub>3</sub> pH 7.4. C4 is thawed on ice and diluted 1:2000 in B1 buffer (Barbital (16 mM), NaCl (580 mM), CaCl<sub>2</sub> (8 mM), MgCl<sub>2</sub> (4 mM) pH 7.4. Cat. No. 210050 Bie Berntsen). Pre-diluted rMBL:MASP complex and the test samples are diluted 1:10 in the C4:B1 buffer and a dilution series of 2-fold dilutions are prepared.

**[0199]** The mannan coated plate is placed on ice and the samples are applied onto the plate. The reaction is initiated when the samples are transferred to the 37 degrees Celsius incubator.

**[0200]** The plate is incubated for 90 min at 37 degrees Celsius in a controlled incubator and washed three times in TBST/Ca<sup>2+</sup> buffer (Tris (10 mM), NaCl (150 mM), Tween-20 (0.05%w/v), CaCl<sub>2</sub> (100 mM) pH 7.4) in a plate washer. 100 µl per well of biotin-labelled human anti C4 antibody (Goat anti-human C4, from DAKO)(1 µg/ml) diluted 1:1000 in TBST/Ca<sup>2+</sup> buffer is added and incubated for 90 min at 25 degrees Celsius in a heating tower. Plates are washed three times in TBST/Ca<sup>2+</sup> buffer in a plate washer. 100 µl per well of Europium-streptavidin solution (Wallac 1244-360) is diluted 1:1000 in Europium dilution buffer (Tris (10 mM), NaCl (150 mM), Tween-20 (0.05%), EDTA (25 µM)) and incubated for 60 min at 25 degrees Celsius in a heating tower. Plates are washed three times in TBST/Ca<sup>2+</sup> buffer in the plate washer. 100 µl Enhancement solution (Wallac 1244-105) is added per well and the samples shaken for 5 min in a plate shaker. Samples are settled for approximately 5 min and inserted into a Wallac Victor 2<sup>d</sup> Multicounter 1420. The fluorescence of Eu<sup>3+</sup> is measured using the standard Europium program.

**[0201]** The data obtained from the reading of the assay should be plotted as follows:

**[0202]** The MBL concentration at the x-axes and the Europium counts (the cpm) at the y-axes. The MBL concentration should be pictured as the natural log whereas the Europium should be the linear data.

**[0203]** For the data analysis and EC50 calculations use the Hill coefficient equation and 4 parameters.

**[0204]** The relative potency of the rMBL sample is determined from the rMBL reference standard as follows:

$$\text{Relative potency} = (\text{EC50reference standard}/\text{EC50test}) * 100$$

#### Example 10

##### **[0205]** Size exclusion chromatography (SEC)

All samples for analysis should be free of visible precipitates and prepared in HPLC vials using caps with septa. The protein solution is applied to the Superose column at room temperature in a Tris-based buffer (pH=7.4).

**[0206]** The Superose 6 HR10/30 column is delivered pre-packed from Amersham Biosciences.

Chromatography is performed according to the below specification:

Resin Type	Superose 6
Resin Size	Area 0.78 cm <sup>2</sup> , Height 30 cm
Temperature	25°C
Solvent Flow	38 cm/hr (0.5 mL/min) during the complete chromatography
Gradient Profile	Isocratic; 40 mL elution buffer per run, corresponding to a runtime of 80 mins.
Detection	280 nm (detection af protein) Sensitivity: AuxiliaryRange=2, Range=1, Response=4 (important!)
Run Time	80 min
Sampling Frequency	5 Hz (each 0.2 s)

Solvents		
Elution buffer (STOCK)	Tris (0.1 M) NaCl (1.4 M) NaN <sub>3</sub> (0.01 % w/v) pH=7.4 (20°C)	Tris (24.2 g), NaCl (163.6 g) and 2.0 mL natriumazid (10%), are dissolved in H <sub>2</sub> O (ad 2000 mL). During the process, pH is adjusted with 5M HCl. Estimated storage time: Long
Elution buffer	Tris (10 mM) NaCl (140 mM) NaN <sub>3</sub> (0.001 % w/v) pH=7.4 (20°C)	Elutionbuffer(STOCK) (200 mL) is diluted into water (ad 2000 mL). pH in the final solution should be controlled. Estimated storage time: Medium
Regeneration buffer (Acid)	HCl (0.1 M)	HCl (5M, 10mL) is dissolved in H <sub>2</sub> O (ad 500 mL). Estimated storage time: Long
Regeneration buffer (Alkaline)	NaOH (0.5 M)	NaOH (20 g) is dissolved in H <sub>2</sub> O (ad 1000 mL). Estimated storage time: Long

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## SEQUENCE LISTING

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# Claims

1. A pharmaceutical composition comprising pharmaceutically acceptable additives and

a) at least 200 µg/ml protein containing material, wherein mannan binding lectin (MBL) and/or MBL variants constitutes at least 35% (w/w) of the total protein; or

b) at least 400 µg/ml mannan binding lectin (MBL) and/or MBL variants

2. The composition according to claim 1, wherein the composition is a protein solution.

3. The composition according to claim 1, wherein the composition is a protein suspension.

4. The composition according to any of claims 2 and 3, wherein the solution or suspension is frozen.
5. The composition according to claim 1, wherein the composition is a dry composition, comprising freeze-dried protein.
6. The composition according to claim 5, wherein said dry composition is capable of being reconstituted into a solution.
7. The composition according to any of claims 1.a), 3 and 6, wherein said solution or suspension is suitable for administration by injection or infusion.
8. The composition according to any of claims 1.a), 3 and 6, wherein said solution or suspension is suitable for administration as bolus injection.
9. The composition according to any of claims 1.a), 3 and 6, wherein said solution or suspension is suitable for administration by intravenous injection or infusion.
10. The composition according to any of claims 1.a), 3 and 6, wherein said solution or suspension is suitable for administration by subcutaneous injection.
11. The composition according to any of claims 1.a), 3 and 6, wherein said solution or suspension is suitable for administration by intramuscular injection.
12. The composition according to claim 1, wherein the composition comprises at least 750 µg/ml protein containing material or salts thereof.
13. The composition according to claim 1, wherein the composition comprises at least 750 µg/ml MBL or MBL salts.
14. The composition according to claim 1, wherein the composition comprises in the range from 200 to 3000 µg/ml MBL and/or MBL variants.
15. The composition according to claim 1, wherein MBL or MBL salts constitutes at least 50% (w/w) of the total protein.
16. The composition according to claim 1, wherein the total protein furthermore comprises one or more MBL associated serine protease (MASP) or fragments thereof.
17. The composition according to claim 16, wherein MASP is selected from the group consisting of MASP-1 of SEQ ID NO:2, MASP-2 of SEQ ID NO:3, MASP-3 of SEQ ID NO:4, fragments thereof and functional equivalents thereof.
18. The composition according to any of the preceding claims, wherein MBL is selected from the group consisting of MBL of SEQ ID NO: 1, fragments thereof and functional equivalents thereof.
19. The composition according to claim 18, wherein MBL is purified naturally occurring human serum MBL.
20. The composition according to claim 18, wherein MBL has been recombinantly produced.
21. The composition according to claim 20, wherein more than 50% of MBL is bigger than 200 kDa as determined by non-reducing SDS-PAGE.
22. The composition according to claim 1, wherein at least one pharmaceutically acceptable additive is an isotonic agent.
23. The composition according to claim 1, wherein at least one pharmaceutically acceptable additive is a buffer.
24. The composition according to claim 1, wherein at least one pharmaceutically acceptable additive is a stabiliser.
25. The compositions according to claim 1, wherein the composition furthermore comprises a divalent cation.
26. The composition according to claim 25, wherein the divalent cation is Ca<sup>2+</sup>

27. The composition according to claim 7, wherein the pharmaceutically acceptable additives comprise one or more selected from the group consisting of isotonic salt, hypertonic salt, buffer and stabilisers.
28. The composition according to claim 5, wherein the pharmaceutically acceptable additives comprise one or more selected from the group consisting of isotonic agents, buffer, stabilisers and cryoprotectant agents.
29. The composition according to claim 5, wherein the pharmaceutically acceptable additives comprise glucosemonohydrate, glycine, NaCl and polyethyleneglycol 3350.
30. The composition according to any of claims 23, 25 and 28, wherein the buffer is capable of buffering a solution to a pH in the range of 6 to 8.
31. The composition according to any of claims 23, 25 and 28, wherein the buffer is selected from the group consisting of TRIS, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine buffer.
32. The composition according to claim 22, wherein the composition is isotonic, hypotonic or hypertonic.
33. The composition according to any of claims 22, 25 and 28, wherein the isotonic agent is selected from the group consisting of NaCl, CaCl<sub>2</sub>, KCl, MgCl<sub>2</sub>, mannitol and glycerol.
34. The composition according to any of claims 24 to 28, wherein the stabiliser is selected from the group consisting of poloxamers, Tween-20, Tween-40, Tween-60, Tween-80, metalions, amino acids, polyethylene glucol, Triton, EDTA and ascorbic acid.
35. The composition according to claim 28, wherein said cryoprotectant agent is selected from the group consisting of dextran, polyethyleneglycol, sucrose, trehalose and mannitol.
36. The composition according to claim 7, wherein the composition has been sterilised.
37. The composition according to claim 7, wherein the composition has been subjected to a virus reduction step.
38. The composition according to claim 1, wherein the composition does not comprise protein containing stabilisers.
39. The composition according to claim 1, wherein the composition does not comprise detergents.
40. The composition according to claim 1, wherein the composition does not comprise stabilisers.
41. A pharmaceutical composition comprising pharmaceutically acceptable additives; and mannan binding lectin (MBL) and/or MBL variants; and at least one divalent cation.
42. The composition according to claim 41, wherein the divalent cation is Ca<sup>2+</sup>
43. The composition according to claim 41, wherein the concentration of mannan binding lectin (MBL) and/or MBL variants is at least 1 mg/ml.
44. A process for preparation of a pharmaceutical composition according to any of claims 1 to 43, comprising the steps of
  - a) Providing mannan binding lectin (MBL) and/or MBL variants
  - b) Mixing said MBL and/or MBL variants with pharmaceutical acceptable additives and optionally with divalent cations
  - c) And thereby obtaining a pharmaceutical composition according to any of claims 1 to 43
45. Use of a composition according to any of claims 1 to 43, for the preparation of a medicament for the treatment of a clinical condition in an individual in need thereof.
46. Use according to claim 45, wherein the clinical condition is an infection.

47. Use according to claim 45, wherein the individual is a human being.

48. Use according to claim 45, wherein the individual is a human being suffering from an increased risk of acquiring an infection.

49. Use according to claim 45, wherein the individual is a human being with subnormal serum MBL level.

50. A medicament for the treatment of a clinical condition in an individual in need thereof comprising a composition according to any of claims 1 to 43

51. The medicament according to claim 50, wherein the clinical condition is an infection.

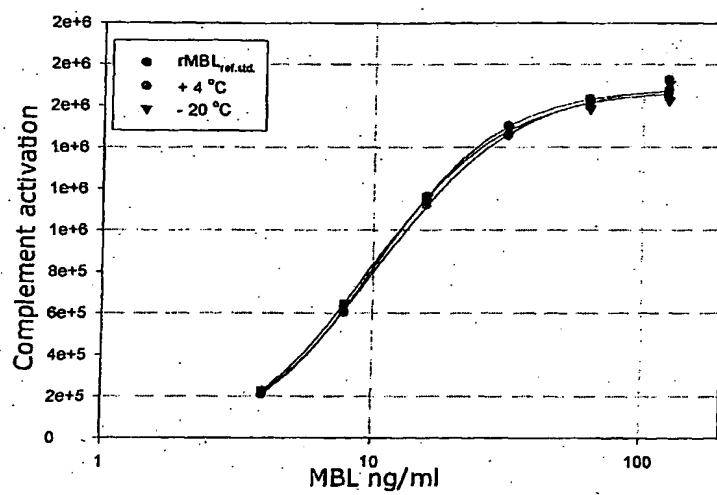
**Fig. 1**

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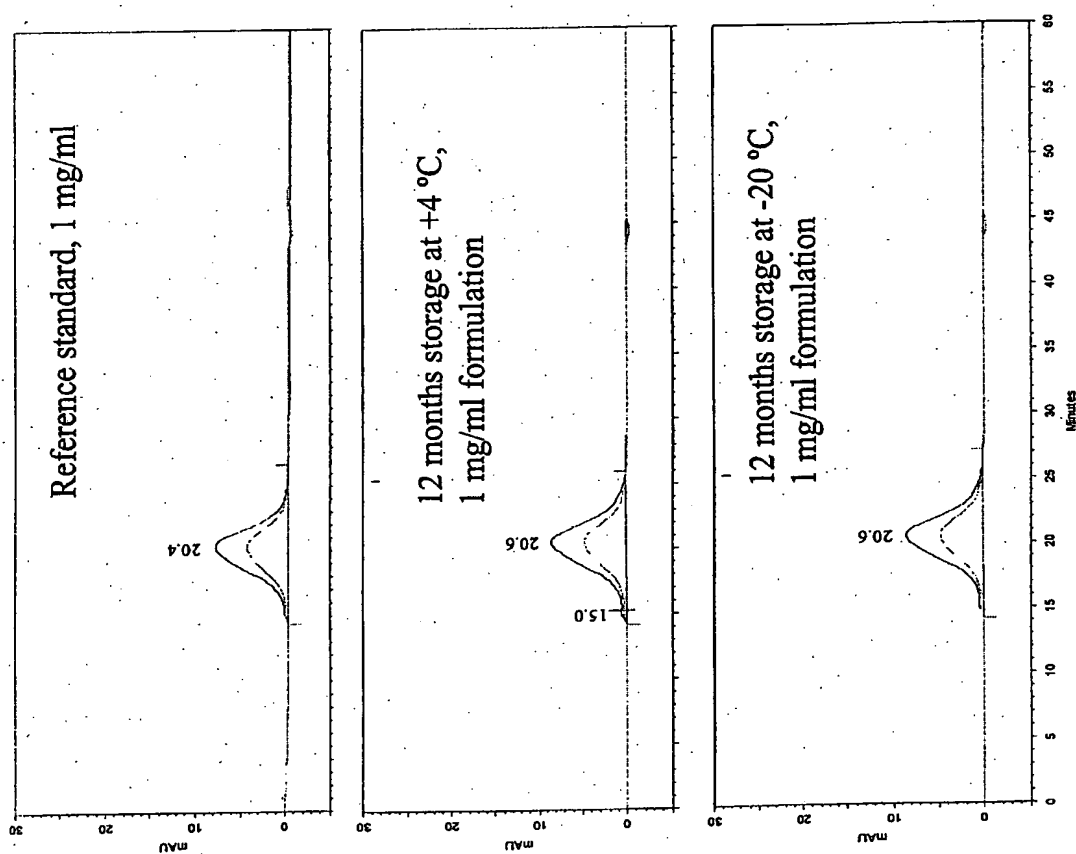
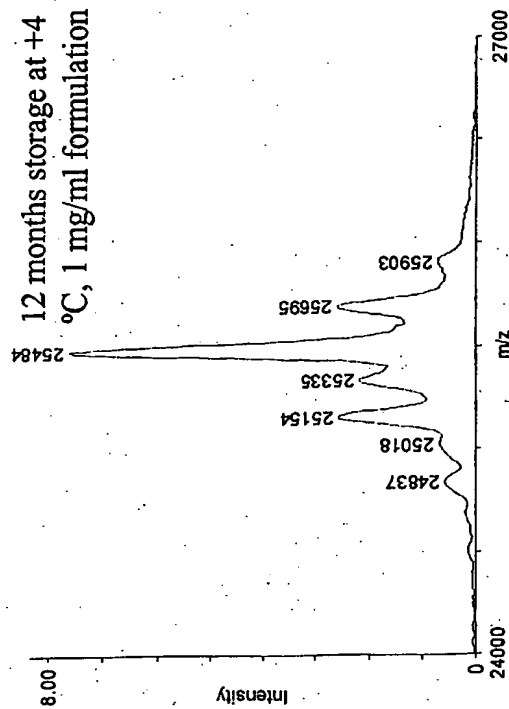
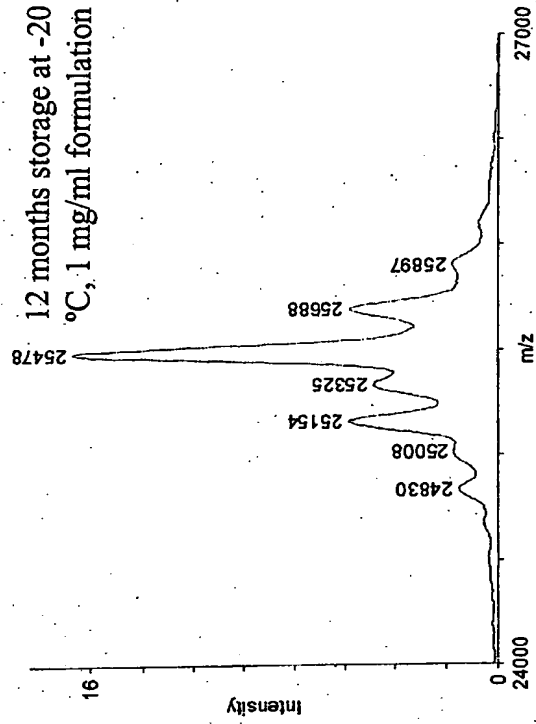
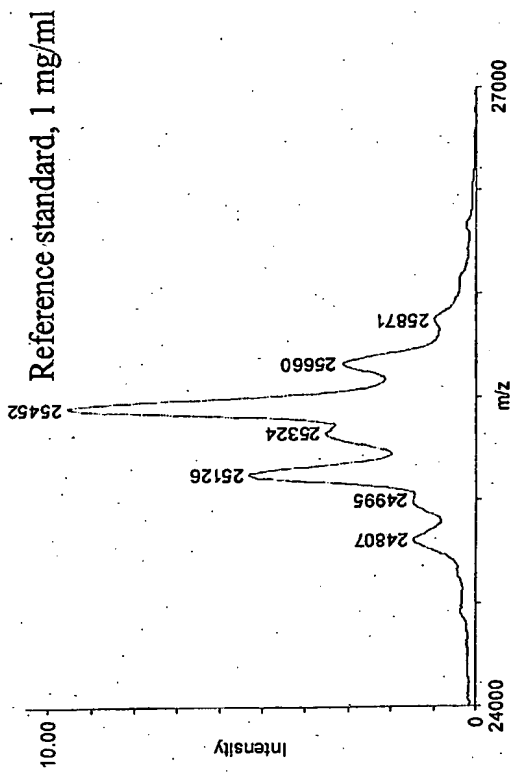


Fig. 3







European Patent  
Office

# PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 03 38 8016

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.C1.7)
X	WO 99 64453 A (STATENS SERUMINSTITUT ;LAURSEN INGA (DK)) 16 December 1999 (1999-12-16)	1-9,12, 14,15, 18,19, 21-25, 29,30, 36-39, 41,42, 44-51	A61K38/17 A61K38/48 A61P31/00 //(A61K38/48, 38:17)
Y	* page 1, line 6 - line 12 *  * page 15, line 11 - line 16 * * page 17, line 1 - line 15; claims * --- -/--	10,11, 16,17, 34,35, 37,38,43	
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INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search: see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		30 June 2003	Bayrak, S
CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>	
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			

EPO FORM 1503 03.92 (P04C07)



European Patent  
Office

INCOMPLETE SEARCH  
SHEET C

Application Number  
EP 03 38 8016

Claim(s) not searched:  
claims 1-18,21-51 (all partially)

Reason for the limitation of the search:

Present claims 1-18,21-51 relate to a compound defined by reference to a desirable characteristic or property, namely "fragment or functional equivalent thereof", "MBL variants", or "protein containing material". The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to a pharmaceutical composition of mannan-binding lectin, or mannose-binding protein, or mannan-binding protein for the therapy of infections; and with due regard to the general concept of the invention.

## PARTIAL EUROPEAN SEARCH REPORT

Application Number  
EP 03 38 8016

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.CI.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	VALDIMARSSON H ET AL: "RECONSTITUTION OF OPSONIZING ACTIVITY BY INFUSION OF MANNAN-BINDINGLECTIN (MBL) TO MBL-DEFICIENT HUMANS" SCANDINAVIAN JOURNAL OF IMMUNOLOGY, BLACKWELL SCIENCE PUBL., OXFORD, GB, vol. 48, no. 2, August 1998 (1998-08), pages 116-123, XP000911946 ISSN: 0300-9475	1-4, 7, 9, 12-14, 18-33, 36, 39-42, 44-51	
Y	* page 117, column 1, line 44 - line 48 *  * page 117, column 2, line 28 - line 32 * * page 118, column 2, line 29 - line 54 * -----	10, 11, 16, 17, 34, 35, 37, 38, 43	TECHNICAL FIELDS SEARCHED (Int.CI.7)

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 38 8016

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

30-06-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9964453 A	16-12-1999	AU 4134399 A	30-12-1999
		CA 2334696 A1	16-12-1999
		WO 9964453 A1	16-12-1999
		EP 1084143 A1	21-03-2001
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		NZ 509089 A	25-10-2002
		US 6429192 B1	06-08-2002
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EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82